

Figure 1:

Amino acid sequences of Cpn60 and Cpn10:

SEQ ID No 1: Cpn10 (encoded by nucleotides pos. 458-751 of Figure 2):

MKIRPLHDRIVVRRKEEETATAGGHILPGAAAEPNQGVVISVGTGRILDNGSVQALA
VNEGDVVVFGKYSGQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 2: Cpn60 (encoded by nucleotides pos. 800-2446 of Figure 2):

MAAKDVLFGDSARAKMLVGVNILADAVRVTLGPKGRNVVIEKSGAPHTKDGVSVA
AREIELKDKFENMGAQMVKEVASQANDQAGDGTTATVLAQAIISGLKSVAAGMNP
PMDLKRGIDKATAAVVAAIKEQAQPCLDTKAIAQVGTISANADETVGRLIAEAMEKV
GKEGVITVEEGKGLEDELVVEGMQFDRGYLSPYFINNQEKMVTMEMENPLILLVDKK
IDNLQELLPILENAVKSGRPLLIVVAEDVEGQALATLVVNNLRGTFKVAAVKAPGFD
RRKAMLQDLAILTGGQVISEELGMSLETADPSSI.GTAKVVIDKENTVIVDGAGTEAS
VNTRVDQIRAEIESSTSVDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGVVAGGGVALIRALSSVTVVGDNEDQNVGIALALRAMEAPIRQI
AGNAGAEGSVVVVDKVKGSGTGSFGFNASTGEYGDMIAMGILDPAKVTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGGMPGMM

Figure 2:

SEQ ID No 3: DNA coding for Cpn60 and Cpn10;

Cpn10, pos. 458-751

Cpn60, pos. 800-2446

cacaaccegaagccatggcggatgcgcctgtgaagaaggcgctggggatgcctgataatggcgcatgggtggaaatggcgg
gtatgcctggcatgtatcactttgtgattcattgtccctgatctgcctaccgtgtaaaaaagatcaggcgtcaaggcgtctctataaaaag
ccgtatcttgtatgagtgttgtttttctgtgtaaaaacgacattctggagtgccggcttttttgttggcataaaaatcagaataatgtgttaatt
ttatgttaactagctggccatataalgtttagttccctgggtggcatgatctcatggfaccttcaactaaggcctgaltcactgct
gttttaacagtaaaataatacgcacgttagaaacataataagcgtatggcattatgaagacggcgtgcatttaattcagatc

Figure 3:

SEQ ID No 4: Amino acid sequence of esterase cloned from *Oleispira antarctica* (EstRB8):

EstRB8 (encoded by nucleotides 1145 to 2143 Frame 2 of Figure 4) 333 aa

MKNTILKSSSRFSLKQLGTGALISSLFFGGCTTQQDNLYTGVMSLARDSAGLEVKTA
SAGDVNLTYMERQGSDKDNAESVILLHGFSADKDNWILFTKEFDEKYHVIAVDLAG
HGDSEQLLTTDYGLIKQAERLDIFLSGLGVNSFHAGNSMGGAIASIYSLSHPEKVSL
TLIDAAGVDGDESEYYKVLAEGKNPLIATDEASFYRMGFTMTQPPFLPWPLRPSLL
RKTLARAEINNKIFSMDMLKTKERLGMTNFQQKIEVKMAQHPLPTLIMWGKEDRVLD
VSAAAFAKKIIPQATVHIFPEVGHLPMVIEIPSESAKVYEEFLSSIK

Figure 4:

SEQ ID No 5: DNA fragment from plasmid pBK1Est coding for esterase of *Oleispira antarctica* (EstRB8):

Nucleotide positions 1-100 correspond to reverse complement of positions 1196-1121 and 3799-3939 correspond to reverse complement of 1043-952 of p8K-CMV vector (Stratagene).

Positions 101-105 are *Bam*HI-*Sau*3A1 fusion and positions 3795-3798 are *Sau*3A1-*Bam*HI-fusion.

Figure 5:

Amino acid sequences expressed from vector pBK1CpnEst: - the co-expression of fragments encoding native chaperonines with the esterase gene (EstRB8), all from *Oleispira antarctica*

SEQ ID No 6: cpn10 (nucleotides 113 to 403; Frame 2 of Figure 6) 97 aa:

MKIRPLHDIRVRRKEEETATAGGIILPGAAAEPNQGVVISVGTGRILDNGSVQALA
VNEGDVVVFGKYSQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 7: cpn60 (nucleotides 455 to 2098; Frame 2 of Figure 6) 548 aa:

MAAKDVLFGDSARAKMLVGVNILADA
RVTLGPKGRNVVIEKSGAPHTKDGVS
V
AREIELKDKFENMGAQMVK
EVASQANDQAGDGT
TTATVLAQAIHSEGLKSVAAGMN
PMDLKRGIDKATAAVVAA
IKEQAQPCLDTKAIAQVGT
ISANADETVGRLIAEAMEKV
GKEGVITVEEGKG
LEDEL
DVVEGMQFD
RGYLS
PYFINNQE
KMT
VEMENPL
LVDKK
IDNLQELL
PILE
VAKSGRPLL
LIV
AED
VEGQAL
ATLV
VNN
LRT
FKV
AAV
KAP
FGD
RRK
AMLQDL
AILTGG
QV
ISEEL
GMS
LETADP
SSIL
GTASK
VV
VID
KENT
VIV
DGAG
TEAS
VNTR
VDQ
IRAE
IES
STS
SDY
DIE
KL
QER
VAK
LAGG
VAV
IKV
GAG
SEM
EM
KEK
DRVD
DAL
HAT
RAA
VEEG
VV
AGGG
VAL
IRAL
SSV
T
V
V
GD
NED
QNV
GIAL
L
R
A
M
E
API
RQI
AG
NAG
AEG
SVV
VDK
V
K
SGT
GS
FG
FN
A
ST
GEY
GDM
IA
M
G
I
L
D
PA
K
V
TR
S
L
Q
AA
AS
I
AG
L
M
I
T
E
A
M
V
A
D
A
P
V
E
E
G
A
G
G
M
P
D
M
G
G
M
G
M
P
G
M
M

SEQ ID No 8: estRB8 (nucleotides 2579 to 3577; Frame 2 of Figure 6) 333 aa:

MKNTLKSSSRFLKQLGTGALISSLFFGGCTTQQDNLYTG
VMSLARDSAGLEV
KTA
SAGDVNLTYMERQGSDKDNA
ESVILLHGFSADKDN
WILFTKEFDE
KYH
VIAV
DLAG
HGDSEQLL
TTDYGLIK
QAERL
DIFL
SGL
GVNSF
HAGNS
MGG
AISAI
YSL
SHPE
KV
SL
TL
IDAAG
V
DGD
TE
SE
YY
K
V
LAE
G
K
N
P
LI
AT
D
E
A
S
F
E
Y
R
M
G
F
M
T
Q
P
P
F
L
P
W
P
L
R
P
S
L
R
K
T
L
A
R
A
E
I
N
N
K
I
F
S
D
M
L
K
T
K
E
R
L
G
M
T
N
F
Q
Q
K
I
E
V
K
M
A
Q
H
P
L
P
T
L
I
M
W
G
K
E
D
R
V
L
D
V
S
A
A
A
F
K
K
I
P
Q
A
T
V
H
I
F
P
E
V
G
H
L
P
M
V
E
I
P
S
E
A
K
V
Y
E
E
F
L
S
S
I
K

Figure 6:

SEQ ID No 9: pBK1CpnEst: - the fusion of native chaperonine-coding fragments with esterase of *Oleispira antarctica* (EstRB8)

The DNA fragment coding for Cpn10 and Cpn60 is flanked by *SacI* site (pos. 69-75) and *Sall* site (encoded by pos. 2138-2143 of Figure 7):

Nucleotide positions 1-75 correspond to reverse complement of positions 1196-1121 and positions 5233-5273 correspond to reverse complement of 1043-952 of pBK-CMV vector (Stratagene)

Small letters -- the Cpn10-Cpn60 encoding fragment,

Capital italics – fragments of vector pBK-CMV

Capital letters – fragment coding for EstRB8 from plasmid pBK1Est

AATCGCAGTGGGTTCTTGTTCATCAACAGCAACAAACGTGAAATACCCCGTA
ATCGCATTTCTGATTATCAAATACATACTTTCCACCAGCATATTAACCTCAAC
TTTAAACTCGTCCGCCCTACCTCTATAACACTGGCAGTCAATTGACAATGGTAC
CTGCGGAACAGGATGCTTAAATCGATTCGATCACTGCTGACGGTTACGATGCT
TTGTCGAGAAAAACGAGTCGCTGCAATAAAAGAAACCTCATCCACTGCATT
GCAGTGCCACCGAATAACGTATCATGATGATTGTTGCTCTGAAATACCGCTT
AGAAATAGTGGTTTGATACCGCTTCGCTGCGCAATAATATCTTCTGCTAA
GAGTGCAGATGGCATACATAACTCGCTTGATTAAGATTAATAATAAGTTA
ACAGTATATTGAACTGAGGGTCTGAAGAACTCTAATAACCTCTGAAGAACCTTGAG
GCCGCTAGAGAGAAAAGACCAGTGATAATATTICATCTGCCATGAGAGCTTATC
ATGAAAGCCTGTGCTTAAATCAATCATTATATTICATCTTAATTGAAATAA
TACCAATATATTICATATATAATTICACACTACCCCTATCTCACTAGACTTCCCCGC
GCATAGCGCAAACAATCAACGCAAGTCACAATAAAGCGGTTGCTGCAACAC
ATGCCCTAGCGTCTAAAGTAGCACGCACAACACTGGCCAGTCGTACTAGCCCCCT
TGCATTGTCAGACGAGCAACAAGCGCTATTAAACTTACCTAAATTCTAACCC
ACCACCAATTGGTTCTTCCACAAACTCAAAAAACTCGTCAAATCCGCTGCAATT
TAAACGCGATGACATAGATCTAATCGATTATCAAACCGCATTCAAGCGCTCATT
AAAAACGCACCACTGGCAAGAAGTCTACCTGCACTGACCAATATGCAAGCGGC
GGCGGAAGAGCTGCCTTGATCGATCAAGAAGAAGGGAGCAGCAAAGAGGAAA
ACAATCAAAAAGAGGAGAGCAATCAAATAAAACGAGTTATIGAGGATTAAAT
TTTAAACAGGTATATTAAATACCCCTCTCGTAGTAAACAATGACTGTATTACAC
AAAAATAAAATAGAGGTATACCATGTCAAACATCTGGTTGAAGTACCAAAGATTG
AAGTATTAAACCGTCAAATGGAAAATACTGCCCTGCAGCAACTTAGGCATTCAAAT
TACAGAAATTGGCGATGATTATATCACTGGCACAATGCCAGCAGATGCACGTACC
TTCCAGCCAATGGGACTGATTCTGGGGCTCAAATGTATTGCTGGCAGAAACAC
TGGGCAGCATGGCAGCTAATGCTGTATTAAATTGCTCAAGAATATTGTGTTGG
CCAAGAAATIAACGCCAACACATACGCCGTGTCGTCGGCATAGTGAUTGGC
ACAGCAACGCTAGTACACAAAGGAAGAACCTCCCAGATTGGAAATTGCAATC
GTTAACGATCCAAGAATTCAAAGCTCTCGAGAGTACTTCTAGAGCGGCCGCGGG
CCCATCGATTITCCACCCGGTGGGGTACCGAGTAAGTGTACCCAATTGCCCTATAGT
GAGTCGTATTACAATTCACTGGCCGTGTTTAC

Figure 7:

Amino acid sequences expressed from vector pBK1CpnSREst: - the co-expression of the stabilized single ring mutant chaperonin with the esterase gene (EstRB8) from *Oleispira antarctica* (cpn10::stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala::est)

SEQ ID No 10: cpn10 (nucleotides 113 to 403; Frame 2 of Figure 8) 97 aa:

MKIRPLHDIRVVRRKEEETATAAGGILPGAAAEEKPNQGVVISVGTGRILDNGSVQALA
VNEGDDVVVFGKYSGQNTIDIDGEELLILNESDIYGVLEA

Below – ***Capital bold letters*** are the mutations introduced

SEQ ID No 11: stabilized single ring mutant of cpn60 (nucleotides 455 to 2098; Frame 2 of Figure 8) 548 aa:

MAAKDVLFGDSARAKMLVGVNILADAVRVTLGPKGRNVVIEKSGAPHTKDGVS
AREIELKDKFENMGAQMVKEVASQANDQAGDITATVLAQAIISGLKSVAAGMN
PMDLKRGIDKATAAVVAAIKEQAQPCLDTKAIAQVGTISANADETVGRLIAEAMEKV
GKEGVITVEEGKGLEDELVVEGMQFDRGYLSFYFINNQEKMVTMEMENPLILLVDKK
IDNLQELLPILENVAKSGRPLLIVVAEDVEGQALATLVVNNLRGTFKVAAVKAPGFD
RRKAMLQDLAILTGGQVISEELGMSLETADPSSLGTASKVVIDKENTVIVDGAGTEAS
VNTRVDQIRAEIESSTSVDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGVVAGGGVALIRALSSVTVVGDNEDQNVGIALALRAMEAPIRQI
AGNAGAAGAIVVVDKVKSGTGSFGFNASTGEYGDMIAMGILDPAKVTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMPGMM

SEQ ID No 12: EstRB8 (nucleotides 2579 to 3577; Frame 2 of Figure 8) 333 aa:

MKNLKKSSSRFSLKQLGTGALISSLFFGGCTTQQDNLYTGVMISLARDSAGLEVKTA
SAGDVNLTYMERQGSDKDNAESVILLHGFSAADKDNWILFTKEFDEKYHVIAVDLAG
HGDSEQLLTDYGLIKQAERLDIFLSGLGVNSFHAGNSMGGAIASIYSLSHPEKVKS
TLIDAAGVGDTESEYYKVLAEGKNPLIATDEASFEYRMGFTMTQPPFLPWPLRPSLL

SN 10/575,505
Replacement Sheet

RKTLARAEINNKIFSDMLKTKERLGMTNFQQKIEVKMAQHPLPTLIMWGKEDRVLD
VSAAAASFKKIIPQATVHIFPEVGHLPMVEIPSESAKVYEEFLSSIK

Figure 8:

SEQ ID No 13: DNA sequence of vector pBK1CpnSREst: the expression cassette for the co-expression of the stabilized single ring mutant chaperonin with the esterase gene (EstRB8) from *Oleispira antarctica* (cpn10::stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala::est)

Nucleotide positions 1-75 correspond to reverse complement of positions 1196-1121 and positions 5233-5273 correspond to reverse complement of 1043-952 of pBK-CMV vector (Stratagene)

DNA fragment coding for Cpn10 and Cpn60 is flanked by *SacI* site (pos. 69-75) and *Sall* site (pos. 2138-2143).

In the DNA sequence:

Small letters -- the Cpn10-Cpn60 coding fragment,

Capital italics – fragments of vector

Capital letters – fragment coding for EstRB8 from plasmid pBK1Est

Capital bold letters = introduced mutations

CTTCAAAAAATAATTCCACAAGCAACTGTCATATTTCTGAAGTAGGCCAC
CTACCTATGGTAGAAATTCTAGTGAAAGCGCTAAAGTTATGAAGAGTTTGT
CCTCTATTAAATAAGAGCACATAATCATGACTGACTTATAAACAGCCAAGCATT
AAAATGCTTGGCTGTTATTTAATGCCAAATTATTCAACGACCAAGCTGCG
GTAAAATCGCAGTGGGTTCTGTTTCAACAGCAACAAACGTGAAATACCC
CGTAATCGCATTTCTGATTATCAAATAACATACTTCCACCAAGCATATTAACTT
CAACTTTAAACTCGCCGCCACCTCTATAACACTGGCAGTCATTGACAATG
GTACCTGCCGGAACAGGATGCTTAAATCGATTGATCACTGCTGACGGTTACGA
TGCTTGTGAGAAAAACGAGTCGCTGCAATAAAAGAAACCTCATCCATCCACTG
CATTCGAGTGCCACCGAATAACGTATCATGATGATTGTTGTCTGGAAATACC
GCTTAGAAATAGTGGTTTGATACCGCTTCGCTGCGCAATAATATCTCTCT
GCTAAGAGTTCGGATGGCATAACATAACTCGCTGATTAGATTAAATAATAAAT
AGTTAACAGTATATIGAACTGAGGGCTGAAGAACTCTAACCTCTGAAGAACT
TTGAGGCCGCTAGAGAGAAAAGACCAAGTGATAATATTCACTTGCATGAGAGC
TTATCATGAAAGCCTGTGCTTAAATCAATCATTATATTATCATTCTTAAATTGA
AATAATACCAATATATTCAATATAATTCAACACTACCCATTCTCACTAGACTT
CCCGCGCATAGCGCAAACAATCAACGCAAGTCACAATAAGCGGTTGCGCTGC
AACACATGCCCTAGCGTCTAAAGTAGCACGCACAACACTGGCCAGTCGTACTAGC
CCCTTGCATTGTCAGACGAGCAACAAGCGCTATTAAACTACCTAAATTTC
TAACCACCAATTGGTTCTTCCACAAACTCAAAAAACTCGTCAAATCCGCTTG
CAATTAAACCGCATGACATAGATCTAATCGATTATCAAACCCGATTCAAGCGC
TCATTAAAAACGCAACTGGCAAGAAGTCTACCTGCACTGACCAATATGCAAG
CGGCGCGAAGAGCTGCCTTGATCGATCAAGAAGAAGGGGACCAAGGAGG
AAAACAATCAAAAAGAGGAGAGCAATCAAATAAAACGAGTTATTGAGGATT
AATTAAACAGGTATATTAAATACCCCTCTCGTAGTAAACAATGACTGTATT
CACAAAAATAATAGAGGTATACCATGTCAAACATCTGGTTGAAGTACCAAAG
ATTGAAGTATTAAACCGTCAAATGGAAAATCTGCCTGCAGCAACITAGGCATT
AAATTACAGAAATTGGCGATGATTATATCACTGGCACAATGCCAGCAGATGCACG
TACCTTCCAGCCAATGGGACTGATTCAATGGCGGCTCAAATGTATTGCTGGCAGAA
ACACTGGGCAGCATGGCAGCTAACTGCTGTATTAAATTGTCAGAAATATTGTG
TTGGCCAAGAAATTAAACGCCAACACACAGCGGTGTTGCTCCGGCATAGTGAC
TGGCACAGCAACGCTAGTACACAAAGGAAGAACCTCCAGATTGGGAAATTG
CATCGTTAACGATCCAAAGAATTCAAAGCTCTCGAGAGTACTTCTAGAGCGGCCG

SN 10/575,505
Replacement Sheet

CGGGGCCCATCGATTTCCACCCGGGTGGGGTACCGTAAGTGTACCCAATTGCCCT
ATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTAC

Figure 9:

Amino acid sequence of the stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala of Cpn60:

SEQ ID No 14: Cpn10 (nucleotides 458-751 of Figure 10):

MKIRPLHDIRVRRKEETATAGGIILPGAAAEPNQGVVISVGTGRILDNGSVQALA
VNEGDVVVFGKYSQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 15: Cpn60 (nucleotides 458-751 of Figure 10):

MAAKDVLFGDSARAKMLVGVNILADA
RVTLGPKGRNVVIEKSF
GAPHTKDGVS
V
AREIELKDKFENMGAQMVK
EVASQANDQAGD
GTTATVLAQAI
HSEGLKSVAAGMN
PMDLKRG
IDKATAAVV
AAIKEQA
QPCLDTKA
IAQVGT
ISANADE
TVGRLIA
EAMEKV
GKEGV
ITVEEGKG
LEDEL
DVVEGMQ
FDRG
YLS
PYFINN
QEKM
TVEMEN
PLILL
VDKK
IDNLQ
ELLPILE
NVA
KSGRPL
LIV
AED
VEG
QAL
ATLV
VNN
LRGT
FKV
AAV
KAP
FGD
RRK
AMLQ
DLAIL
TGG
QVISE
ELGMS
LETADP
SSILGT
ASKV
VIDK
ENTV
IVDG
AGTEAS
VNTR
VDQ
IRAE
IESST
SDY
DIEKL
QERV
AKLAG
GVAV
IKVG
AGSEM
MEMKE
KKDR
VD
DAL
HAT
RAA
VEEG
VVAG
GGVAL
IRAL
SSVT
VVG
DNED
QNV
GIAL
RAL
RAME
APIR
QI
AGN
AGA
AAV
VDK
VKSG
TGS
FGFN
AST
GEY
GDM
IAM
GILD
PAK
VTR
SSL
QAA
ASI
AGL
MIT
TEAM
VAD
APVE
EGAG
GMP
DMGG
GGM
GGMP
GMM

Figure 10:

SEQ ID No 16: DNA sequence of the stabilized single ring mutant

Glu460Ala/Ser462Ala/Val463Ala;

In the DNA sequence:

Small letters – the Cpn10-Cpn60 coding fragment.

Big bold letters = introduced mutations

ggtaacgcaggcgtgCagggGcagCggtgtataaagtgaaatctggcacaggtagcttgttaacgccagcacaggtagatggcgatatgatgcgtgggtatttgtacccctgcggaaaagtcacgcgttcatctctacaagccgcggcgctatcgaggttgatgatcacaaccgaageccatggtgtgcggatgcgcctgtgaagaaggcgctggggtaigccgtataggccggcatgggtggaatggcggtatgcctggcatgtatgtaaactacttgtgattcatgtctgateigctaccgtgtaaaaagatcaggcteaaggctgtctataaaaagccgtatcttgtaigagtggtgtttctgtgaaaacgcacattggagtgcggcttttgtatittgtatataaaattcagaatatttgtattttgtaactagctggccataatgttgtagttcctctgggtggcatgtctcatggacttcaactaagecctgattcactgcgttttaacagtaaaataacgcacgtagaaacataataagcgtatggeattaatgaagacggctgcaittaaatcagaatc